

# An Optimized Set of Human Telomere Clones for Studying Telomere Integrity and Architecture

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Telomere-specific clones are a valuable resource for the characterization of chromosomal rearrangements. We previously reported a first-generation set of human telomere probes consisting of 34 genomic clones, which were a known distance from the end of the chromosome (~300 kb), and 7 clones corresponding to the most distal markers on the integrated genetic/physical map (1p, 5p, 6p, 9p, 12p, 15q, and 20q). Subsequently, this resource has been optimized and completed: the size of the genomic clones has been expanded to a target size of 100–200 kb, which is optimal for use in genome-scanning methodologies, and additional probes for the remaining seven telomeres have been identified. For each clone we give an associated mapped sequence-tagged site and provide distances from the telomere estimated using a combination of fiberFISH, interphase FISH, sequence analysis, and radiation-hybrid mapping. This updated set of telomeric clones is an invaluable resource for clinical diagnosis and represents an important contribution to genetic and physical mapping efforts aimed at telomeric regions.

## Introduction

The telomeric regions of human chromosomes are enriched for CpG islands and genes and are believed to have the highest gene density in the entire genome (Saccone et al. 1992). Characterization of telomeric regions is important for our understanding of the relationship between chromosome structure and function (Flint et al. 1997a) and because chromosomal rearrangements involving telomeres result in a number of clinical conditions, including mental retardation (Knight et al. 1999; Holinski-Feder et al. 2000), recurrent miscarriage and hematological malignancies (Tosi et al. 1999). The ability to identify each chromosome end also aids in the characterization of known chromosomal abnormalities (Ning et al. 1996; Horsley et al. 1998). However, telo-

meric rearrangements are a challenge to detect using conventional clinical cytogenetics methods, since most terminal bands are G-band negative. Thus, alternative methodologies, such as fluorescence in situ hybridization (FISH) or comparative genomic hybridization (CGH) array-based approaches, are required for high-resolution screening of telomeric regions. Furthermore, for mapping and sequencing efforts, it is imperative that the location of the telomere be known so that maps can be anchored and telomeric closure achieved.

The size and complexity of telomeric regions has made them extremely difficult to analyze at a molecular level. The high degree of sequence similarity between subtelomeric domains on different chromosomes, the presence of genes in subtelomeric DNA, and chromosomal rearrangements occurring within subtelomeric repeats all have important implications for screening strategies aimed toward assessment of subtelomeric integrity (Brown et al. 1990; Rouyer et al. 1990; Wilkie et al. 1991; Flint et al. 1997b; Monfouilloux et al. 1998; Trask et al. 1998). If chromosome unique (and, hence, more centromeric) sequences are used for screening, then rearrangements telomeric to the probe will be missed. If repetitive subtelomeric DNA sequences are used, then the exact chromosomal origin of the detected DNA will be uncertain. A practical solution to this problem is to use unique sequence probes, accepting

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that a small proportion of rearrangements may be missed.

The discovery that telomeres from human chromosomes can be cloned by functional complementation in yeast enabled the molecular characterization of subtelomeric repetitive and chromosome unique DNA (Brown et al. 1989; Cross et al. 1989; Riethman et al. 1989; Bates et al. 1990). When yeast artificial chromosome (YAC) vectors with only one functional telomere are ligated to human genomic DNA, clones survive in the yeast host cells if they are stabilized by the presence of a second telomere derived from the human genomic DNA ("half-YACs") (Xiang et al. 1999). Characterization of half-YAC libraries produced in this way has resulted in the development of a valuable set of molecular resources for analyzing most, if not all, chromosome ends (National Institutes of Health et al. 1996; Rosenberg et al. 1997). As part of an international collaboration to identify a complete set of human telomere probes for use in FISH assays, we previously reported a first generation set of 41 clones consisting of 34 telomere clones that were a known distance from the end of each chromosome and 7 clones corresponding to the distal marker from the integrated map (1p, 5p, 6p, 9p, 12p, 15q, 20q) (National Institutes of Health et al. 1996). The total number of clones targeted for the complete set was 41 rather than 48, since no efforts were made to identify clones representing the short arm of the acrocentric chromosomes, and clones corresponding to the X and Y pseudoautosomal regions are shared. Most of the clones in the initial set were cosmid clones (35–40 kb), which are adequate in FISH experiments as a single probe, but frequently do not produce a sufficiently robust signal in multicolor experiments. In addition, some clones showed cross-hybridization to other chromosome regions by FISH. Seven chromosome arms were previously represented by distal markers, which were an unknown distance from the end of the chromosome. Subsequently, we have shown that clones corresponding to such distal markers can be as far as 1 Mb from the telomere, thus increasing the possibility that smaller telomeric rearrangements may be missed (Lese et al. 1999).

For these reasons, and to contribute to genome mapping and sequencing efforts, the first-generation telomere set has been refined. All cosmid probes have been replaced with more robust PAC, P1, or BAC clones and probes previously derived from the most distal markers on the Whitehead/MIT map have been converted into clones of known distance from the end of the chromosome. We describe here the isolation and detailed characterization of this second-generation set of human telomere clones.

## Material and Methods

### *Isolation of Second Generation Set of Telomere-Specific Clones*

PCR screening was carried out using sequence-tagged sites (STSs) developed from distal markers, telomeric sequence information, half-YAC vector-insert junction sequences or end sequences from telomeric clones. The methods for identifying half-YAC clones, isolating vector-insert junction fragments and obtaining sequence information have been described elsewhere (Cross et al. 1989; Riethman et al. 1989; Negorev et al. 1994), as have the method employed to make STSs from bacterial clones (Rosenberg et al. 1997). The software package Primer 3 was utilized to create primers from sequence information (see Electronic-Database Information). Primer pairs were tested and optimized on a monochromosomal hybrid panel (available from the Coriell Institute for Medical Research or from the United Kingdom Human Genome Mapping Project resource center) to identify conditions that produce chromosome specific PCR amplification for screening purposes. Standard PCR conditions with 1.5 mM MgCl<sub>2</sub> and a 55°C annealing temperature were used for all newly developed STSs.

A (TTAGGG)<sub>n</sub> telomere probe and the STSs were used to screen PAC and BAC libraries (PAC library from Genome Systems and the Bacpac Web site [RPCI-1]; BAC libraries from Genome Systems and Research Genetics [CITB-978SK-B and CITB-HSP-C]). The letters RG or GS preceding a clone address identify that clone as being isolated from the Research Genetics library and the Genome Systems library, respectively. The presence of an STS in positive clones was confirmed by PCR amplification of the STS from a single colony of the PAC, P1, or BAC clone. A second hybridization screen confirmed (TTAGGG)<sub>n</sub> positive clones.

### *BAC End Sequencing*

BAC DNA was isolated using an automated nucleic acid isolation system (AutoGen 740, Integrated Separation Systems) and purified with Microcon 100 columns (Amicon). One microgram of BAC DNA and 40 pmol of T7 and SP6 primers were used for sequencing with the ABI Prism™ Big-Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems). The sequences of the T7 and SP6 primers and the sequencing reaction times have been previously reported (Matsumoto et al. 1997). Sequence analysis was completed on an ABI 377 automated DNA sequencer.

### *FISH Characterization/Testing of New Telomere Probes*

DNA was isolated for FISH analysis using an Autogen 740 or a standard alkaline-lysis protocol (Sambrook et al. 1989). FISH analysis was carried out on normal metaphases cultured from peripheral blood to test each new probe identified. Probe and slide preparation, DNA hybridization, and analysis were carried out using methods described elsewhere (Buckle and Rack 1993; Chong et al. 1997). At least 10 cells were analyzed using direct microscopic visualization and digital-imaging analysis to verify probe location and chromosome specificity. Inverted DAPI staining was used to obtain a G-banded pattern for chromosome identification. A probe was characterized as unique only if the signal was telomere specific (i.e., no cross-hybridization to any other chromosomes), both directly visualized at the microscope and on the individual fluorochrome raw image. Probes were tested for common polymorphisms by analyzing the size and intensity of the FISH signals on at least five unrelated individuals.

### *Interphase FISH Analysis*

Interphase distance measurements were carried out as described elsewhere (Lese et al. 1999). Briefly, dual-color FISH was used for measuring genomic distances between differentially labeled clones in interphase nuclei from G<sub>0</sub> fibroblast cells. The distances measured were from the telomere clone of interest to a subtelomeric clone known to be contained within the relevant half-YAC. Actual genomic distances were calculated using the measure length command contained in the IP Lab Spectrum software package (version 3, Signal Analytics); the measure length command was calibrated using images from a Zeiss stage micrometer. Genomic distances were derived from a calibration curve that was produced in our laboratory (Lese et al. 1999). For each telomere, 50–100 interphase distances were measured.

For the telomeric region of 1p, interphase FISH mapping was performed using three-color ordering. FISH was carried out as described above. Probes were labeled with Spectrum Orange, biotin, or digoxigenin, and detection was carried out using avidin-Cy5 (for biotin-labeled probes) and anti-digoxigenin-FITC (for digoxigenin-labeled probes). Spectrum Orange-labeled nucleotides are directly incorporated into the DNA, and therefore antibody detection of was not necessary. The three labeled DNA probes were hybridized simultaneously to interphase cells from a chromosome 1 monochromosomal hybrid cell line (GM13139A, Coriell Institute for Medical Research). Images were captured using ViewPoint software (Vysis). Only cells that showed three hybridization signals in a linear formation were used for clone ordering. At least 30 cells were scored to

determine the order that occurred with the greatest frequency.

### *Sequence Analysis*

M13 bacteriophage libraries were created from the 5p, 6p, 12p, and 20q (TTAGGG)<sub>n</sub> positive PACS and were screened by hybridization and sequenced as described elsewhere (Flint et al. 1997a, 1998). After *Alu* and other repetitive DNA were excluded, the sequences obtained from the clones were screened against dbEST and EMBL with BLASTN, and against a nonredundant compilation of Swissprot, PIR and wormpep (*Caenorhabditis elegans* genes) with BLASTX. The BLAST outputs were filtered using MSPcrunch (Sonnhammer and Durbin 1994), requiring ≥90% identity for dbEST and EMBL matches. BLASTN/MSPcrunch also was used to identify sequence matches between telomeres.

### *Radiation Hybrid (RH) Mapping*

RH mapping makes use of a panel of somatic cell hybrids, with each cell line containing a random set of fragments of irradiated human genomic DNA in a hamster background. To obtain mapping data, our STSs were screened by PCR against the Genebridge4 radiation-hybrid screening panel at the Whitehead Institute for Biomedical Research/MIT Center for Genome Research. The STSs submitted for RH mapping are listed in table 1. The PCR results were recorded as RH vectors of 1's and 0's, indicating the presence or absence, respectively, of the STS in each hybrid. The most likely chromosomal assignments and placements of the STSs were determined from the vector data by computational analysis. Two markers were considered to be linked if they had vectors of statistically significant similarity and if a measure of their separation was obtained from the analysis of the degree of difference between the two vectors. Map distances were calculated in units of centiRays (cR) (referring to the X-ray dosage used to construct the RH panel).

### *FiberFISH*

PAC DNAs were nick-translation labeled with digoxigenin-11-dUTP (Boehringer Mannheim) according to a standard protocol (Buckle and Rack 1993). To obtain the (TTAGGG)<sub>n</sub> fluorescent probe, PCR was carried out in a 40-ml reaction containing 1 ng telomere probe (a plasmid containing 3 kb of canonical telomere sequence, kindly supplied by Dr. N. Royle), 1 mM oligonucleotide Tel1 (5'-TTAGGGTTAGGGTTAGGG-3'), 1 mM oligonucleotide Tel2 (5'-CTAACCCTAACCCTAACC-3'), 1 × PCR Buffer (10 × PCR +Mg stock, Boehringer Mannheim), 1 × dNTPs (100 × DNA polymerization mix, Pharmacia Biotech) and 4 U *Taq* polymerase (Boehringer Mannheim). The PCR conditions were 30 cycles

of 96°C for 40 s, 65°C for 40 s, 70°C for 5 min. The products (300 bp–12 kb in size) were then purified using the Wizard PCR kit (Promega) and nick-translation labeled with biotin-11-dUTP (Boehringer Mannheim). For each slide to be hybridized, ~60 ng labeled telomere probe and ~60 ng labeled PAC DNA were vacuum dried with 15–20 mg COT-1 (Gibco-BRL), and the pellet was resuspended in 45 ml hybridization solution (65% formamide [Fluka], 1 × SSC, 10% dextran sulfate [molecular weight 500,000; BDH, Inc.]). DNA fibers were prepared on poly-L-lysine coated slides (Sigma), as described by Heiskanen et al. (1996). The prepared slides were washed in 2 × SSC for 5 min and then were dehydrated through a 70%, 90%, and 100% ethanol series for 3–5 min at each percentage. The slides were equilibrated on a 37°C hotblock, and the probe solution was added and was sealed in place with a 22-mm × 64-mm cover slip. The DNA and probes were denatured together on a 75°C hotblock for 1 min 35 s, and the slides were hybridized overnight in a slide box floating in an uncovered 37°C water bath. The slides were washed, and the probes were detected by fluorescently conjugated antibodies, as described elsewhere (Knight et al. 1997). The hybridized DNA fibers were viewed using an Olympus BX60 microscope, as described elsewhere (Knight et al. 1997). The (TTAGGG)<sub>n</sub> signals fluoresced red, and the PAC clone signals fluoresced green. The distances between the red and green signals were estimated on a linear scale, with the average size of a PAC being taken as ~100 kb. To achieve this, the fiberFISH images were saved in TIFF format. They were then accessed using Adobe Photoshop 4.0, and two measurements were recorded in mm: Y, the length of the PAC signal (beginning to end of the green signal, including closely interrupted signals, but excluding any small isolated signals considered to be nonspecific); and X, the intervening distance between the green signal and the red signal. The distance from the most distal end of the PAC to the most proximal telomeric sequence was calculated in kb as 100(X/Y). A schematic representation of the derivation of fiberFISH distances is given in figure 3. To confirm the validity of the fiberFISH results, two control hybridizations using the 4p cosmid, B31, and the 16p cosmid, cGG4, which are known to be ~70 kb and 81 kb, respectively, from their cognate telomeres (Flint et al. 1997a), were also performed.

#### *RecA-Assisted Restriction Endonuclease (RARE) cleavage*

RARE cleavage is a method used to perform sequence-specific cleavage of genomic DNA (Ferrin and Camarini-Otero 1991, 1994). It exploits the ability of the RecA protein of *Escherichia coli* to pair an oligonucleotide to its homologous sequence in duplex DNA and to form

a three-stranded complex. The duplex in the complex is protected from methylation by site-specific DNA methyltransferases. Thus, when the three-stranded complex is exposed to site-specific DNA methyltransferases and the oligonucleotide and RecA protein are subsequently removed, the only sites which can be cleaved by methylation sensitive restriction endonucleases are the sites in the duplex which were previously protected from methylation. The major application of the RARE cleavage technique is in constructing long-range physical maps of genomic DNA, but it has also been adapted so that distances between a particular restriction endonuclease site and the respective telomere can be determined (Ferrin and Camerini-Otero 1994; Riethman et al. 1997).

The RARE results given in table 1 were obtained using the method of Riethman et al. (1997) and have been reported previously (Macina et al. 1994, 1995; Negorev et al. 1994; Reston et al. 1995; National Institute of Health et al. 1996). In brief, sequence information from half-YAC vector-insert junction clones was used to design oligonucleotides that spanned known restriction endonuclease sites present in the insert sequences. The oligonucleotides were then used in RARE analyses of genomic DNA. The cleaved DNA was subjected to pulsed field gel electrophoresis, probed with single-copy sequences distal to the restriction endonuclease site and the distance of the restriction site from the telomere thereby inferred from the size of the hybridizing fragment.

## Results

### *Large-Format Clone Conversion*

The telomere clones identified in this study are shown in table 1, along with the evidence for telomeric localization. The corresponding primer sets used for library screening and newly developed STSs are listed in table 2. The map positions of the clones in relation to the telomere are derived from a combination of sequenced contig results, physical mapping, half-YAC sizes (determined by pulsed field gel electrophoresis), RARE analysis, radiation hybrid mapping of STSs, interphase FISH, and fiberFISH. Where complete sequence is available, the distance between the associated STS and the telomere is given. Since the physical distance from the telomere is not yet known for all STSs derived from half-YACs, the maximum distance given is the size of the half-YAC. However, clones isolated with the half-YAC vector-insert junction are expected to be more centromeric than those isolated with telomeric STSs, since the latter are derived from sequences telomeric to the vector-insert junction.

Figure 1 shows a comparison of metaphase FISH results obtained with a cosmid clone compared to those

Table 1

## Second-Generation Telomere Clones and Their Location

Telomere	Clone ID	Clone Type	Maximum Physical Distance from Telomere (kb)	Method	Fiber-FISH (kb) <sup>a</sup>	RH Mapping (cR) <sup>b</sup>	Marker
1p	GS-62-L8	PAC	200	Interphase mapping from subtelomeric sequences		7.69 tel	1PTEL06
	GS-232-B23	BAC	200	Interphase mapping from subtelomeric sequences			CEB108/T7
1q	GS-160-H23	PAC	80	Contig	<50		1QTEL19
	GS-167-K11	BAC	270	RARE from YAC end sequence			1QTEL10, 1QTEL19, VIJ-YRM2123
2p	GS-892-G20	PAC	330	RARE from YAC end sequence			2PTEL27
	GS-8-L3	BAC	330	RARE from YAC end sequence			VIJ-YRM2052
2q	GS-1011-O17	PAC	240	RARE from YAC end sequence			2QTEL47
	RG-172-I13	BAC	240	RARE from YAC end sequence			VIJ-YRM2112
3p	GS-1186-B18	PAC	450	RARE from YAC end sequence	150–250		3PTEL25
	RG-228-K22	BAC	450	RARE from YAC end sequence			3PTEL25
3q	196-F4	PAC	450	YAC clone size			3QTEL06
	GS-56-H22	BAC	450	YAC clone size			3QTEL05
4p	GS-36-P21	PAC	73	Sequence: Z95704	80–100		4PTEL04
	GS-118-B13	BAC	101	Sequence: Z95704; walk from 4PTEL01			GS10K2/T7
4q	GS-963-K6	PAC	275–500	Contig (vanGeel et al. 1999)		2.9 tel	4QTEL11
	GS-31-J3	BAC	300–700 <sup>c</sup>	Interphase distance from 4QTEL11			AFMA224XH1
5p	GS-189-N21	PAC	Unknown			16.2 cen	5PTEL48
	GS-24-H17	BAC	Unknown	Distal marker of 5p contig			C84C11/T3
5q	GS-240-G13	PAC	245	YAC clone size			5QTEL70
6p	GS-62-I11	PAC	300	Fiber FISH	<300	19.3 cen	6PTEL48
	GS-196-I5	BAC	300				6PTEL48
6q	GS-57-H24	PAC	280	RARE from YAC end sequence			6QTEL54
	GS-164-D18	PAC	255	YAC clone size	~130	5.76 cen	7PTEL03, VIJ-YRM2185
7q	GS-3K-23	PAC	7	Sequence: AF027390			7QTEL20, VIJ-YRM2000
8p	GS-580-L5 <sup>d</sup>	PAC	250	YAC clone size	NP		8PTEL91
	GS-77-L23	BAC	250–450 <sup>c</sup>	Interphase distance from 8PTEL91			AFM197XG5
8q	GS-489-D14	PAC	170	RARE from YAC end sequence			8QTEL11
	GS-261-I1	BAC	170	RARE from YAC end sequence			VIJ-YRM2053
9p	GS-43-N6	PAC	600	YAC clone size	<600	13.9 tel	9PTEL30
	RG-41-L13	BAC	190	YAC clone size			305J7-T7
9q	GS-112-N13 <sup>d</sup>	PAC	65	YAC clone size	NP		9QTEL33
	GS-135-I17	BAC	65	YAC clone size			VIJ-YRM2241
10p	GS-306-F7	PAC	320	YAC clone size	~100		10PTEL45
	GS-23-B11	BAC	320	YAC clone size			10PTEL006
10q	GS-137-E24	PAC	270	YAC clone size	~100		10QTEL24
	GS-261-B16	BAC	270	YAC clone size			10QTEL24
11p	GS-908-H22 <sup>d</sup>	PAC	125	YAC clone size	NP		11PTEL03
	GS-44-H16	PAC	125	YAC clone size			VIJ-YRM2209
11q	GS-770-G7 <sup>d</sup>	PAC	65	YAC clone size	NP		11QTEL38
	GS-26-N8	PAC	65	YAC clone size			VIJ-YRM2072
12p	GS-496-A11 <sup>d</sup>	PAC	Unknown	TTAGGG clone	NP	27.8 cen	12PTEL27
	GS-8-M16 <sup>d</sup>	BAC	100	YAC clone size			TYAC-14
12q	GS-124-K20	BAC	100	YAC clone size			8M16/SP6
	GS-221-K18	PAC	190	RARE from YAC end sequence	100/600 <sup>e</sup>		12QTEL87, VIJ-YRM2196
13q	GS-163-C9	PAC	170	RARE from YAC end sequence	<20		13QTEL56
	GS-1-L16	PAC	170	RARE from YAC end sequence			VIJ-YRM2002
14q	GS-820-M16	PAC	200	RARE from YAC end sequence			14QTEL01
	GS-200-D12	BAC	200	RARE from YAC end sequence			VIJ-YRM2006
15q	GS-124-05 <sup>d</sup>	PAC	300 <sup>e</sup>	Interphase mapping from 15q YAC	NP	24.46 tel	15QTEL56
	GS-154-P1	PAC	300 <sup>e</sup>	Interphase mapping from 15q YAC			WI-5214
16p	GS-121-I4	PAC	160	Sequence: Z84721			16PTEL05
	RG-191-K2	BAC	160	Sequence: Z84721			16PTEL05
16q	GS-240-G10	PAC	200	YAC clone size		17.91 tel	16QTEL48
	GS-191-P24	PAC	200	YAC clone size			16QTEL13
17p	GS-202-L17 <sup>d</sup>	PAC	60	Contig (Xiang et al. 1999)	30/100 <sup>e</sup>		17PTEL80
	GS-68-F18	BAC	100–200	Walk from 17PTEL80			282M15/SP6
17q	GS-362-K4 <sup>d</sup>	PAC	90	YAC clone size	NP		17QTEL13
	GS-50-C4	BAC	100–300	Interphase distance from 17QTEL13			AFM217YD10

**Table 1 (Continued)**

Telomere	Clone ID	Clone Type	Maximum Physical Distance from Telomere (kb)	Method	Fiber-FISH (kb) <sup>a</sup>	RH Mapping (cR) <sup>b</sup>	Marker
18q	GS-964-M9	PAC	290	RARE from YAC end sequence			18QTEL11
	GS-75-F20	BAC	290	RARE from YAC end sequence			VIJ-YRM2050
19p	GS-546-C11	PAC	250–500	LLNL chromosome 19 contig	NP	16.13 tel	19PTEL29
	RG-129-F16	BAC	250–500	LLNL chromosome 19 contig			129F16/SP6
19q	GS-48-O23	PAC	250–500	LLNL chromosome 19 contig		11.65 tel	19QTEL12
	GS-325-I23	BAC	250–500	LLNL chromosome 19 contig			D19S238E
20p	GS-1061-L1	PAC	180	YAC clone size			20PTHY33
	GS-82-O2	PAC	180	YAC clone size			20PTEL18
20q	GS-81-F12 <sup>d</sup>	PAC	50	Fiber FISH	<50	28.2 tel	20QTEL14
21q	GS-63-H24	PAC	175	RARE from YAC end sequence			21QTEL07
	GS-2-H14 <sup>d</sup>	P1	175	RARE from YAC end sequence			VIJ-YRM2029
22q	GS-99-K24 <sup>d</sup>	PAC	120	Sequence: (Dunham et al. 1999)	150–200	12.6 tel	22QTEL31
	GS-3018-K1	BAC	155	Sequence: (Dunham et al. 1999)			3018K1/T7
XpYp	GS-98-C4	PAC	490	STS map (Nagaraja et al. 1997)			DXYS28
	GS-839-D20	BAC	160	STS map (Nagaraja et al. 1997)			DXYS129
XqYq	GS-225-F6	BAC	100				CDY16C07
Xq	GS-202-M24	PAC	500	STS map (Nagaraja et al. 1997)			DXS7059

<sup>a</sup> NP = not possible, because of cross-hybridization.

<sup>b</sup> cen = centromeric; tel = telomeric.

<sup>c</sup> The distance given is the sum of the size of the relevant half-YAC and the estimated interphase FISH distance between the BAC clone and a subtelomeric clone known to be contained within the half-YAC.

<sup>d</sup> Cross-hybridization visible by FISH; see table 3 for details.

<sup>e</sup> Fiber-FISH indicates a size polymorphism.

obtained with a BAC clone. As is evident, the BAC clone produces a larger hybridization signal that is more easily visualized than the cosmid clone. Images for other telomere BAC, PAC, and P1 clones can be viewed at the University of Chicago Department of Human Genetics Web page. The PAC polymorphism and cross-hybridization profiles are provided in table 3. The 2q PAC (1011-O17) still detects the common polymorphism encountered previously, but the stronger hybridization signal reduces the likelihood of misinterpretation compared to the original 2112b2 cosmid. The XpYp PAC (98-C4) does not contain the polymorphism previously encountered with the first-generation cosmid. Overall, the larger clones are more robust for multiplex FISH assays where more than one telomere clone is used per hybridization area. Larger-format clones are also better suited for other genomewide scanning methods, such as comparative genome hybridization (CGH) to microarrays, where larger insert sizes are expected to give improved signals because there are more sequences to which the labeled target DNA can hybridize.

#### New Telomere Clones

New telomere clones for 1p, 5p, 6p, 9p, 12p, 15q, and 20q were isolated using multiple strategies. To identify a telomeric clone for 1p, we first generated an STS

from the cosmid clone CEB108 (National Institutes of Health et al. 1996) and identified large insert clones, including the BAC clone GS-232-B23. We then used three-color interphase FISH to determine the relationship of this BAC clone with respect to the telomere. This was achieved by use of two other clones, PAC GS-63-M14 and BAC GS-145-I23. GS-63-M14 is derived from the 8p telomere but contains subtelomeric sequences with homology to 1p (observed as cross-hybridization by metaphase FISH) and GS-145-I23 is derived from the 17q telomere but shows cross-hybridization to multiple telomeres, including 1p. The cross-hybridization of GS-145-I23 to multiple telomeres indicates that it is more telomeric than GS-63-M14, which only cross-hybridizes to 1p (Flint et al. 1997b). Hence, for the three-color interphase FISH, GS-145-I23 served as the telomeric anchor to determine the order and distance of GS-232-B23 with respect to GS-63-M14. By using a monochromosomal hybrid cell line containing only human chromosome 1, the cross-hybridization signals from the other subtelomeric repeat sequences were eliminated, thus allowing a more clear interpretation. As shown in figure 2, interphase ordering showed tel-GS-145-I23-GS63M14-GS232B23-cen. The genomic distance between GS-63-M14 and GS-232-B23 was estimated to be <200 kb using interphase FISH measurements on G<sub>0</sub> fibroblasts.

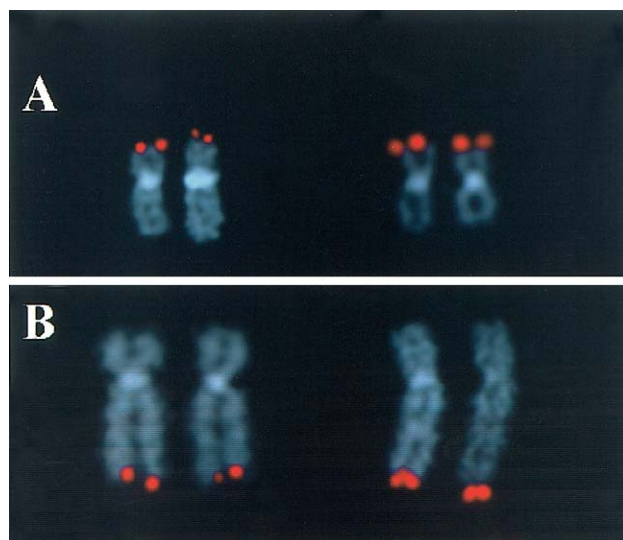
Table 2

## STSs for Telomere Clones

CHROMOSOME	MARKER	REFERENCE	PRODUCT SIZE (bp)	PRIMER (5'→3')		CLONES
				Forward	Reverse	
1p	1PTEL06	This article	121	AGTCTGAAGGTGACAGCGGT	AGTGCTCGGAGCCTGGA	GS-62-L8
1p	CEB108/T7	This article	104	GCCTTTAAACAGAGACTGCGG	GAGGAGGAGGAAGGTTGAGG	GS-232-B23
1q	1QTEL19	D1S3739	156–189	GGAGTTAAGGTTGAAGAGCC	TTCACGTACAACAGTATCTC	GS-160-H23
1q	1QTEL10	D1S3738	120	GATCCATTCTGTATGATGG	ACTCATTCTACGAAGTCAGC	GS-167-K11
2p	2PTEL27	D2S2983	230–242	TATATACATTAGGAATAATAGGAGT	GTCCTCTCCATGTATAGA	GS-892-G20
2p	VIJ-YRM2052	GenBank U32389	120	GATCTCACTGCAATTTCTACA	TCCATTTTCTCCAAGTTATCA	GS-8-L3
2q	2QTEL47	D2S2983	146–162	GTGAGGTCAGGAACGCTAAG	CTGTCATCGCTATGAGCCAG	GS-1011-O17
2q	VIJ-YRM2112	D2S447	227	AATTTACAGTGGCATTTCAGGC	CACACACAGCAGGGACGAC	RG-172-I13
3p	3PTEL25	D3S4559	182–190	GCTAGCATGAGAATGTATAG	TACGCCTAATGAGATAACAG	GS-1186-B18, RG-228-K22
3q	3QTEL06	D3S1272	262–276	TCACAAGGGAAATAACTGTTTTAC	TTCCTGTAACCCCTCCAAAAT	GS-196-F4
3q	3QTEL05	D3S4560	150–164	TCACAGTGGCCAAGATATCA	TCCATGTTGCTGCAAATGAC	GS-56-H22
4p	4PTEL04	D4S3360	166–182	CTAGCTTTGATTCTATTGACC	GGTCTAAATCAATGACCTAAGC	GS-36-P21
4p	GS10K2/T7	This article	273	TCAGAAGGACTGGGAGATGG	TAGAGTCCAGCCAGAAACCC	GS-118-B13
4q	4QTEL11	This article	239–243	AGGCAGTGAGAAAGGAATGG	TGCGTGTAAGTGTGTGCAAG	GS-963-K6
4q	AFMA224XH1	D4S2930	219	CCTCATGGTAGGTTAATCCCACG	TATTGAATGCCCCCATTTC	GS-31-J3
5p	5PTEL48	This article	219–231	TAATTACCCTTGGCTACAGG	AGGCCTTGGAGGGAAGATTTC	GS-189-N21
5p	C84C11/T3	This article	136	GTTCTCATGTGTGACTCCCCG	GGGGAGACACAGCCCATAG	GS-24-H17
5q	5QTEL70	D5S2097	191	CTATTTTTATTTCAGTTGGCTGTTT	AAGGAAACGTTCTCTAAGTTATTA	GS-40-G13
5q	GS35O8/T7	This article	150	CTGCCTTTTGGGGAAGACC	TATGGAATGGAAGTGGTGCC	GS-240-G13
6p	6PTEL48	This article	268–296	GATAGGCCTAGAACTGTGAG	GTTTGTCCCTTTACCTCCCC	GS-62-L11, GS-196-I5
6q	6QTEL54	D6S2522	206–210	CAGAACAGATTAAAGACTCAG	GCATTTATCAACTTGTGTCC	GS-57-H4
6q	VIJ-YRM2158	GenBank U11835	95	TTTGTGACCAACATTATCAAGG	GCTGTGCAAGTCCCTTGTTC	GS-57-H24b
7p	7PTEL03	This article	132	ACCACCAGGCCTAAAGTGTG	CCGAGCTTAGTGTCCCAGAG	GS-164-D18
7p	VIJ-YRM2185	GenBank G31341	357	CCTGGGTGTGCCGTAAAC	TCACTGAGCTTGCTGGCTTA	GS-164-D18
7q	7QTEL20	This article	217–235	GCCCAGCCTGTGTATGGTTA	CTAAAGGAAGTTCTACAGG	GS-3K-23
7q	VIJ-YRM2000	GenBank G31340	204	CTCAATATCACACAACAGTG	GCTGAAAGGAAAACTGATTG	GS-3K-23
8p	8PTEL91	D8S2333	144	CCCAGCATCTGACATGAGAA	TTATCGCTCAAGAAGTAGAC	GS-580-L5
8p	AFM197XG5	D8S504	200	ACTGGGTCACGAGGGA	CATGCCCATTTTCCAG	GS-77-L23
8q	8QTEL11	D8S1925	94–112	TGAGATCTGTCTCTATACAC	CTACAGGTGTGTGCTACCAT	GS-489-D14
8q	VIJ-YRM2053	GenBank U11829	75	ATTCTCCTATGTTTCCTGGTGC	GTTCAACCACTTCCCACTCTTG	GS-261-I1
9p	9PTEL30	This article	241–245	TCTCCGTCACCTGCACTCCAG	CTGGCAGTGAGCCGAGATCG	GS-43-N6
9p	305J7-T7	GenBank AF170276	128	TTACATTCCCTTTTCATCACC	CACTGCACTCCAGTTTGG	RG-41-L13
9q	9QTEL33	D9S2168	122–130	ATCTGTGTTGGATTCTTGGC	ACTGAACACACCTGTACAGG	GS-112-N13
9q	VIJ-YRM2241	D9S325	90	GGGAGTCCCTACTCTGGAAGC	TGTGAAACTCCTCTACCAGCG	GS-135-I17
10p	10PTEL45	D10S2488	186–198	TGGGTAACAGAGTGAGACTGTCTC	AGTACACTGATGATTTTCATTCCT	GS_306-F7
10p	10PTEL006	GenBank Z96139	167	CTTCTAGCAAAACAGGTTGC	ACTGTCTTTTCATCCTACGC	GS-23-B11
10q	10QTEL24	D10S2490	163	CTCCAAGGGTAACATTGAAC	CGTAAACGTTGTGAGATGGT	GS-137-E24, GS-261-B16
11p	11PTEL03	D11S2071	168–202	AGGGCAATGAGGACATGAAC	ATGTGGCTGGTCCACCTG	GS-908-H22, GS-44-H16
11q	11QTEL38	D11S4974	158	AGGGTCTAAGCTACCCAAAG	TATGCAGGTTACAGTCACG	GS-770-G7
11q	VIJ-YRM2072		83	AGTCTACTCCCTGTTAAGGTAA	CCTGGGCCACCATTTACTAG	GS-26-N8

12p	12PTEL27	This article	209	ATGCCTCAGTTTGTCTTGGC	TGCCCAGAAAGGTTTAACTGG	GS-496-A11
12p	TYAC-14	Vocero-Akbani et al. (1996 )	140	CCTCGGTTTAGCAGAGCAGC	GGGAGAATGGGGTCTCTCC	GS-8-M16
12p	8M16/SP6	This article	333	GTATTGAAATCCAATCTATGCCACC	CTCTCAAGTAATTCATATTCTTTGGG	GS-124-K20
12q	12QTEL87	D12S2343	170–174	TGAGACTGCAGTGAACCATG	TCTTTCTTGAAGAGGAAGCC	GS-221-K18
12q	VIJ-YRM2196	GenBank U11838	116	GATGAGGGAGTTTGGGGG	AAGCCATTT CCACTCCTCCT	GS-221-K18b
13q	13QTEL56	D13S1825	162–166	TTGCAGTGAGCTGATATCGC	TAACAGGATCTCTGTAAAGCG	GS-163-C9
13q	VIJ-YRM2002	D13S327	210	CAGAGGTAGCTTCATAAAG	CTATCTGCAACTTATTTA CC	GS-1-L16
14q	14QTEL01	D14S1420	174–182	GTGCCTGTAGGTATCTATGC	GCTCCCTATTTGCAAGATAC	GS-820-M16
14q	VIJ-YRM2006	D14S308	321	GGCGTTTCTGATGTTTTTAAGC	GAGACGATGGAGGAGTGAGC	GS-200-D12
15q	15QTEL56	This article	145	CCATCTAGCCCCTCATGTTTT	AACTGAAAGCCACCACTTGG	GS-124-05
15q	WI-5214	D15S936	124	GTGGAAAATGATTTCCCTACTG	CATAACACATAACAGGCCCC	GS-154-P1a
16p	16PTEL05	D16S3400	170–182	AGCTGAGATCACGCCATTGC	TGTGAGGAGACAGGAAAGAG	GS-121-I4, RG-191-K2
16q	16QTEL48	This article	94	GCAGAGTTACAGACGGAGGC	TCTGGCCAAAGGGAATAAAA	GS-240-G10
16q	16QTEL013	GenBank Z96319	221	AAAGCTCTCAGAACCTCCCC	AGAGGTTCCCATGTAGTTCC	GS-191-P24
17p	17PTEL80	D17S2199	190	ACGCACATGCTTTCACGAAC	CCTGCCTTAGTGTTCCCTGAG	GS-202-L17
17p	282M15/SP6	This article	225	GTGATGACTGTGGAAGACAGAGGAAG	CACAGTCTCAGAAATATGTTCTCC	GS-68-F18
17q	17QTEL13	D17S2200	233–235	CGTGCCACTCAAATATAAAC	CAAAATAAAAACCTGCAAGCAATATA	GS-36-K4
17q	AFM217YD10	D17S928	150	TAAAACGGCTACAACACATACA	ATTTCCCCACTGGCTG	GS-50-C4
18p	18PTEL02	D18S1389	220	TCATTTATATGAAATTTCAAGGGAT	AAAAATAAAGCAATTGCTCATAGAA	GS-52-M11
18p	VIJ-YRM2102	D18S552	103	GGTAGGAGAGGAGGAAAAAAGC	CTGTCTTTGAGCCAGAAAGTCC	GS-74-G18
18q	18QTEL11	D18S1390	155	CCTATTTAAGTTTCTGTAAAGG	ATGGTGTAACCTGTGGAA	GS-964-M9
18q	VIJ-YRM2050	NIH (1996)	151	GTGCCACGAGAACGTGAAC	ATTCCATCACCTAAAAACATGGC	GS-75F-20
19p	19PTEL29	This article	202	AACAGGAAGACGGGAGTCCT	TGCAGACAACAGCAGGTACC	GS-546-C11
19p	129F16/SP6	LLNL Chromosome 19 map	254	ACTCAGCACCTCCCTCACAG	GTGTTT CCT GCTTCTCTGCC	RG-129-F16
19q	19QTEL12	This article	148	CACTACAGCCTGGCAGATGA	AACAAGCAAAGTCCACCTGG	GS-48-O23
19q	D19S238E	Ashworth et al. (1995)	107	TGCTCCAGGAAATTGGAGTT	CGAAGCACCAAGCTGGTGCA	GS-325-I23
20p	20PTHY33	D20S502	245–261	GGTTCAATGCTACTCAATGGC	AACCACACTGACATCGTAGTGG	GS-1061-L1
20p	20PTEL18	D20S1157	139–141	GATAGTCACTTCAACAGATGG	GTTTGCCAGGCTCACATTTA	GS-82-O2
20q	20QTEL14	This article	161	CCAGCCTAGGTGACAAGAGC	AATGTCAGTGCCTCAACCCT	GS-81-F12
21q	21QTEL07	D21S1575	311–343	GAAACCCATCTCACATGCAG	GAAGTGCTCTAAGAACTTGC	GS-63-H24
21q	VIJ-YRM2029	(Reston et al. 1995)	116	AGGATAATATGTGATGGGCAGG	AGTCTTCTGTGTCCCTCAGCA	GS-2-H14b
22q	22QTEL31	D22S1726	183	TGCATTAGGTAGATGCTGGG	TACACTAGACCCAGGTGAAG	GS-99-K24
22q	3018K1/T7	GenBank AQ093314				GS-3018-K1
XpYp		DXYS28	275	GGAACCTGGCTTTTCATTTCC	AGGTCACCTGGATGGTCACT	GS-98-C4
XpYp		DXYS129	164	ATACTATGCAGTTTAAAGCG	AAGCTGGTGTTCACTGAAA	GS-839-D20
XqYq	CDY16C07	GenBank Z43206	122	AATATGTAGTAGAGGGGGTGG	AAACACTTTTCCCACACTACC	GS-225-F6
Xq		DXS7059	187	TAAATGTCATAGGCCGAAAGAATG	GTACCTAATCGCAAGAAACACTGT	GS-202-M24





**Figure 1** Comparison of FISH results obtained with a cosmid clone compared to a BAC clone for the 19p and 10q telomeres. All probes are labeled with digoxigenin and detected with antidigoxigenin rhodamine (red). A, Hybridization of cosmid clone, cF20643 (left), and BAC clone, RG129F16 (right), corresponding to the 19p telomere. B, Hybridization of cosmid clone, c2136c3 (left), and BAC clone, GS261B16 (right), corresponding to the 10q telomere. In both A and B, notice the difference in signal intensity between the two telomere clones; the BAC clone shows a larger signal compared to that of the cosmid clone.

In our first-generation set of telomere clones, the telomeric region of chromosome 5p was represented by cosmid 84C11 (National Institutes of Health et al. 1996). At that time, no link (such as a half-YAC clone) to the telomere of 5p was available, and, therefore, cosmid 84C11 was utilized, because it was the most distal clone yet identified. Since that report, despite many efforts, we have been unable to find unequivocal evidence of telomeric localization for the chromosome 5p clones; a 5p half-YAC clone has not been identified, and no subtelomeric clones have shown cross-hybridization to the 5p region, which could anchor the telomeric end of the chromosome. Peterson et al. (1999) recently published an integrated physical map for chromosome 5p. They used a somatic cell hybrid panel of 5p, created from patients with 5p deletions, to approximate the location of the 5p telomere. The most distal STS on their map of 5p is derived from the end sequence from cosmid 84C11. Therefore, end-sequence from cosmid 84C11 was used to identify PAC GS24H17; both ends of the cosmid are contained within the PAC clone.

The identification of telomere clones for 9p, 12p, and 15q was previously reported (Lese et al. 1999) and thus are briefly summarized here. STSs designed from half-YAC vector-insert junction sequences were used to isolate clones for the 9p and 12p telomeres. For the 15q

telomere, PAC GS-154-P1 (isolated with distal marker, WI-5214) was shown to be <100 kb from a chromosome 19-derived subtelomeric clone, GS-196-D24. GS-196-D24 was isolated using the vector-insert junction sequence from half-YAC yRM2001, and it contains 15q and other telomeric sequences, observed as cross-hybridization in a FISH assay.

To identify the remaining telomere clones (6p and 20q), a total genomic PAC library was screened by hybridization using an oligonucleotide for the telomeric repeat sequence (TTAGGG)<sub>n</sub>, thereby identifying 75 clones. FISH showed that seven of these were telomere-specific, hybridizing to 5p, 6p, 8p, 10p, 12p, 20q, and 22q. Of the remaining (TTAGGG)<sub>n</sub> positive PACs, 39 hybridized to multiple telomeres, whereas 7 mapped to multiple centromeres and 19 were located in interstitial regions.

Using interphase FISH, the distance of the 6p PAC clone, 62I11, from the most distal marker utilized previously (PAC GS-36-I2, AFMA339YD9) was estimated to be >1.5 Mb. This result demonstrates a substantial gap in the integrated physical and genetic maps for the telomeric region of chromosome 6p. At low-stringency washes, PAC GS-62I-11 showed faint cross-hybridization to the 20q telomere, and, therefore, sequence information from this clone was used for further library screening. This resulted in the isolation of clone GS-196-I5 which hybridized only to the 6p telomere. The distance of four of the new clones from their cognate telomeres (1p, 5p, 6p, and 20q) was unknown and was estimated by RH mapping and fiberFISH.

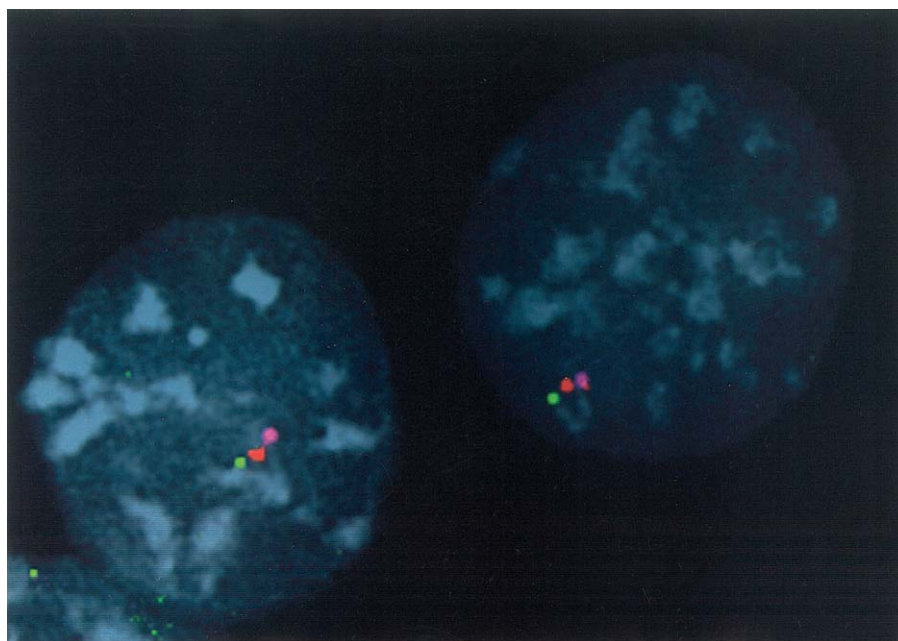
### RH Mapping

RH mapping was used to localize STSs generated from (1) the TTAGGG positive clones (5p, 6p, 12p, and 20q), (2) unmapped first-generation clones, and (3) from un-

**Table 3**

#### Cross-hybridization and Polymorphisms of Second-Generation PACs

Telomere	Probe
Polymorphisms:	
2q	GS-1011-O17
Cross-hybridizations:	
8p with 1p and 3q (both faint)	GS-580-L5
9q with 10p and 16p, 18p, XqYq (faint)	GS-112-N13
11p with 17p	GS-908-H22
11q with 12q (interstitial)	GS-770-G7
12p with 6p and 20q (both faint)	GS-496-A11
12p with 6p and 20q (both faint)	GS-8-M16
15q with 1q (interstitial) and 15q (interstitial)	GS-124-O5
17p with 17q (two interstitial sites)	GS-202-L17
17q with 1p and 6q (both faint)	GS-362-K4
20q with 6p (faint)	GS-81-F12
22q with 2q (interstitial)	GS-99-K24



**Figure 2** Three-color interphase FISH ordering at the human 1p telomere. Interphase cells from a chromosome 1 monochromosomal hybrid cell line were hybridized with three probes from the telomeric region of chromosome 1p. GS-232-B23 is labeled with biotin and detected with avidin-Cy5 (purple), PAC GS-63-M14 is labeled with Spectrum Orange (red) and BAC GS-145-I23 is labeled with digoxigenin and detected with anti-digoxigenin FITC (green). The order of these clones was determined to be tel-GS145I23-GS63M14-GS232B23-cen.

mapped half-YAC derived clones (1p, 4q, 7p, 9p, 15q, 16q, 19p, 19q, and 22q). The results of the radiation-hybrid mapping studies are shown in table 1. All STSs mapped telomeric to the most distal framework markers currently on the RH map with LOD scores >13, with the exception of those from the 5p, 6p, 7p, and 12p PACs.

#### FiberFISH

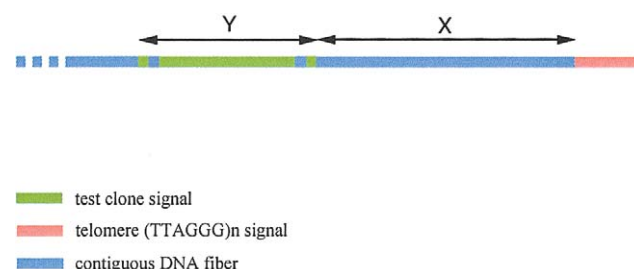
FiberFISH confirmed the telomeric localization of 13 PAC clones and enabled the distance between each of these and the canonical (TTAGGG)<sub>n</sub> repeat to be estimated (table 1). An explanation of the derivation of fiberFISH distances is given in figure 3. An example of the fiberFISH images obtained for the control probes for 4p (cosmid B31) and 16p (cosmid GG4), and an image for the second generation 7p PAC clone is given in figure 4. The control probe for 4p is known to lie ~70 kb from the telomere, and the fiberFISH distance shown in figure 4 was calculated to be ~80 kb. For 16p, the control probe lies 81 kb from the telomere, and the fiberFISH distance shown was calculated to be ~100 kb. These results confirmed the validity of the fiberFISH approach for estimation of the distances between the new telomere clones and their respective telomeres.

At the 12q and 17p telomeres, two different fiberFISH size estimates were obtained on the same DNA sample.

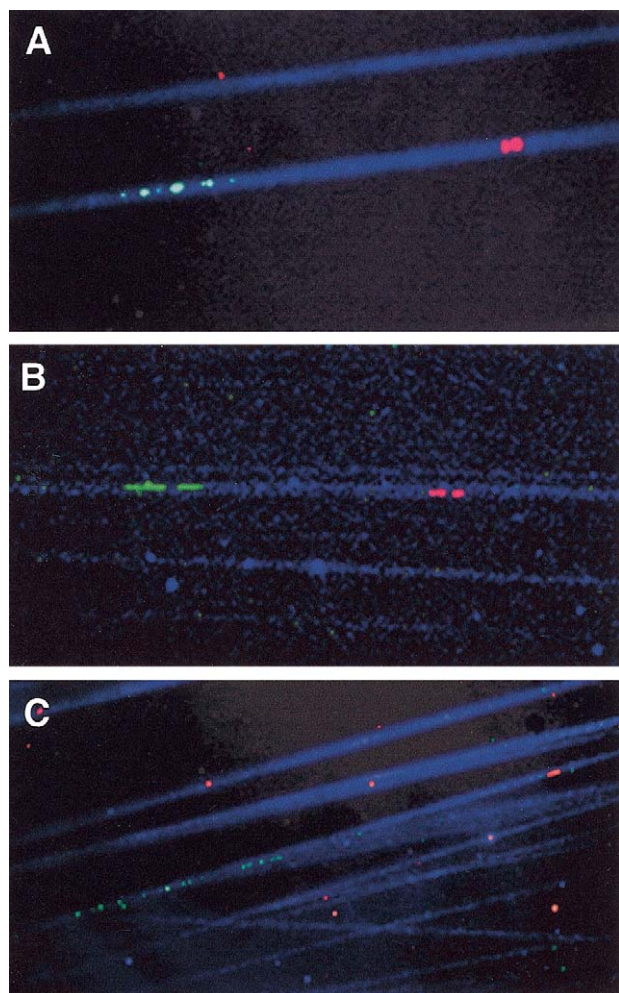
The DNA strands of different lengths occurred at equal frequency on the slide, indicating the presence of an allelic difference. We interpret this as evidence for large-scale size polymorphisms on 12q and 17p, as has already been documented for 16p (Wilkie et al. 1991).

#### Discussion

We have developed a second-generation set of telomere-specific BAC, PAC, and P1 clones that have been char-



**Figure 3** Diagram showing the derivation of fiberFISH distances. The fiberFISH distance in kb ( $D$ ) between the test clone signal and the telomere (TTAGGG)<sub>n</sub> signal is given by  $D = Z(X/Y)$ , where  $Z$  is the known size of the test clone in kb,  $Y$  is the length of the test-clone signal in mm and  $X$  is the distance between the test-clone signal and the telomere signal in mm.



**Figure 4** FiberFISH results showing telomere-specific probe signals in green and (TTAGGG)<sub>n</sub> probe signals in red. A, Control hybridization showing 4p cosmid (B31: ~33 kb insert) ~80 kb from the (TTAGGG)<sub>n</sub> repeat sequence. B, control hybridization showing 16p cosmid (cGG4: ~36 kb insert) ~100 kb from the (TTAGGG)<sub>n</sub> repeat. C, test hybridization showing 7p PAC (164-D18) ~130 kb from the (TTAGGG)<sub>n</sub> repeat.

acterized using a combination of FISH (metaphase, interphase, and fiber), sequence analysis, and RH mapping. A combined approach was taken because no single method of characterization was informative in all situations. With the exception of 5p, telomeric evidence has been provided for each clone. The updated set of human telomere clones provides a resource for investigating the frequency and mechanism of telomeric rearrangements, as well as more detailed biological studies of the architecture and evolution of human telomeres. Telomeric clones are available to the research community via the Bacpac Web site or from Genome Systems or Research Genetics. We have provided a PCR assay for each clone, making it simple to check that the correct clone has been

obtained. The FISH probes are also available commercially through Vysis or Cytocell.

In the absence of accurate physical maps of each telomere, our estimates of the distance from the telomere to each clone are still approximate, but the data indicate that the clones are within 500 kb of each telomere. Where possible, we have mapped clones by fiberFISH. However, in many cases, the clones showed weak cross-hybridization to other chromosomes, making it difficult to discriminate between the cognate signals and those of homologous chromosomes in a fiberFISH assay. In other cases, results were not obtained, either because the (TTAGGG)<sub>n</sub> target sequence was too short to give a clear, interpretable signal (telomere sizes range from 2–20 kb) or because the resolution of the fibers was not sufficiently high. For the majority of cases, the former of these is considered the most likely, since characterization of the same clones (e.g., the 8p and 8q PACs) by alternative methods indicated distances <300 kb from the telomere, a resolution within the means of fiberFISH (table 1). We observed fibers extending beyond the telomere-specific hybridization signals (fig. 4), which we interpret as being caused by fibers from different chromosomal regions remaining together and overlapping as they were pulled across the microscope slide.

We previously reported the characterization of physical gap sizes for 9 human telomeres by measuring the distance between each telomere clone and the corresponding most distal marker (Lese et al. 1999). Gaps in the physical/genetic maps, ranging from <100 kb to >1 Mb, were identified. For clinical testing purposes, these data demonstrate that clones corresponding to distal markers would miss smaller telomeric rearrangements. Thus, clones that are a known distance from the end of the chromosome are the optimal reagents for identifying subtle telomeric rearrangements. Furthermore, the fact that some gaps are ≥1 Mb underscores the importance of more-targeted mapping efforts in these regions to facilitate gene identification and obtain telomeric closure.

As with the first-generation probes, in developing the second-generation set of telomere-specific clones, the need for the clones to be close enough to the telomere to detect rearrangements was balanced with the need for chromosome specificity. Hence, a number of the clones show cross-hybridization with other chromosomes (table 3). With the exception of the cross-hybridization of 9q with 16q, the signals from the homologous chromosomal regions were much weaker than the cognate telomeric signal. The 2q PAC still detects a polymorphism, but the stronger hybridization signal, resulting from the large insert size, reduces the likelihood of misinterpretation in comparison to the original 2112b2 cosmid. For the XpYp clone, the problem of polymorphism was overcome.



The size of the clones (on average 100–200 kb) means that, in general, they provide a stronger FISH signal than does the first-generation set of cosmids. Moreover, they are still small enough to detect rearrangements involving the terminal 200 kb of a chromosome (Wong et al. 1997). The identification of this updated set of human telomere clones provides a valuable new set of tools for clinical diagnosis and for studies of telomere structure and function. Already some of the PACs have been used in conjunction with the first-generation set of clones to confirm that submicroscopic, subtelomeric rearrangements are found in 7.4% of individuals with previously undiagnosed, moderate-to-severe mental retardation (Knight et al. 1999). The clones represent a major step in the generation of molecular tools, which will expedite telomeric gap closure and will help us to elucidate telomere structure and function, ultimately leading to the identification of dosage-sensitive genes involved in human genetic disease.

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## Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Bacpac Web site, <http://www.chori.org/bacpac/home.htm>  
Comprehensive genetic map, <http://www.marshmed.org/genetics/> (for distal marker identification)  
GenBank, <http://www.ncbi.nlm.gov/Genbank/GenbankSearch.html> (for primers and sequence information)  
Généthon map, [http://www.genethon.fr/genethon\\_en.html](http://www.genethon.fr/genethon_en.html) (for distal marker identification)  
Genome Database, <http://gdbwww.gdb.org/> (for primer information)  
Integrated physical map, [http://carbon.wi.mit.edu:8000/cgi-bin/contig/phys\\_map](http://carbon.wi.mit.edu:8000/cgi-bin/contig/phys_map) (for distal marker identification)  
Lawrence Livermore chromosome 19 map, [http://www.bio.llnl.gov/genome/html/chrom\\_map.html](http://www.bio.llnl.gov/genome/html/chrom_map.html) (for chromosome 19p and 19q markers)  
Primer 3, [http://www.genome.wi.mit.edu/genome\\_software/other/primer3.html](http://www.genome.wi.mit.edu/genome_software/other/primer3.html) (for primer development)

TIGR end-sequence database, [http://www.tigr.org/tdb/humgen/bac\\_end\\_search/bac\\_end\\_search.html](http://www.tigr.org/tdb/humgen/bac_end_search/bac_end_search.html) (used to identify 22q telomere BAC clone)

University of Chicago, Department of Human Genetics, <http://www.genes.uchicago.edu> (for additional pictures of human telomere clones)

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